

BIOTECHNICAL APPROACH TO TRACING THE DEVELOPMENT OF OPEN OCEAN DIATOM BLOOMS

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LONG TERM GOALS

This work seeks to develop methods that can identify the genetic affinities among bloom forming diatoms in the ocean. The development of diatom blooms significantly modifies the optical properties of the surface waters yet their occurrence in the open ocean is difficult to predict. Frequently in high latitude oceans, blooms are absent from regions that have abundant nutrients and light. This work focuses on the biological basis for the occurrence of blooms so that the dynamics responsible for their sudden appearance in oceanic regions can be understood and more accurately predicted. An important aspect of this goal is the ability to trace the genetic affinities between coastal and offshore populations to determine the source for the offshore blooms. Such a link would provide the basis for establishing a circulation dependence of diatom blooms in which the biological nature of the upstream regions would exert a strong influence on the bloom forming capabilities of downstream regions.

OBJECTIVES

The primary near-term objective is to implement several basic techniques in DNA analysis that provide some measure of the genetic affinities among diatom sub-populations to determine if these techniques provide sufficient resolution for the spatial analysis. The overall objective actually involved several subcomponents: the collection of a sufficient number of spatially distinct cell lines; the application of appropriate DNA methodologies; and the analysis of the resulting information on genetic affinities. The objectives therefore, required the proper expertise in biological oceanography, taxonomy and molecular biology that are relevant to the long-term goals of the project.

APPROACH

The study was focused on *Chaetoceros contortus*, one of a number of diatoms that is 1) widely distributed; and 2) present in a guild of species that commonly comprises blooms

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in both nearshore and offshore regions. Each of these aspects is important for the hydrographic analysis that will be done. Collaboration with both a diatom taxonomist and someone with experience in molecular techniques as applied to phytoplankton was important to the project. Thus, the taxonomic identification of the source material was made in collaboration with Dr. Jan Rines of the University of Rhode Island and familiarity with the basic molecular techniques used in the project was derived from work in the lab. of Dr. Jane Gallagher at the City College of New York.

The main molecular approach used is restriction fragment length polymorphism (RFLP) analysis of the diatom isolates. This technique was expected to provide the ability to differentiate among closely related subpopulations or races of the same species based on the structural differences that have arisen from insertions/ deletions or other non-lethal modifications in their genomes. Based on the RFLP data, a cladistic analysis is planned in an attempt to determine the cell lines in the collected material. Information about the cell lines can then be compared with the hydrographic characteristics to test the role of horizontal advection in dispersing coastal diatom populations to the open ocean.

WORK COMPLETED

Diatoms have been collected from both culture material and field work to produce a collection from different environments from around the world (fig. 1).

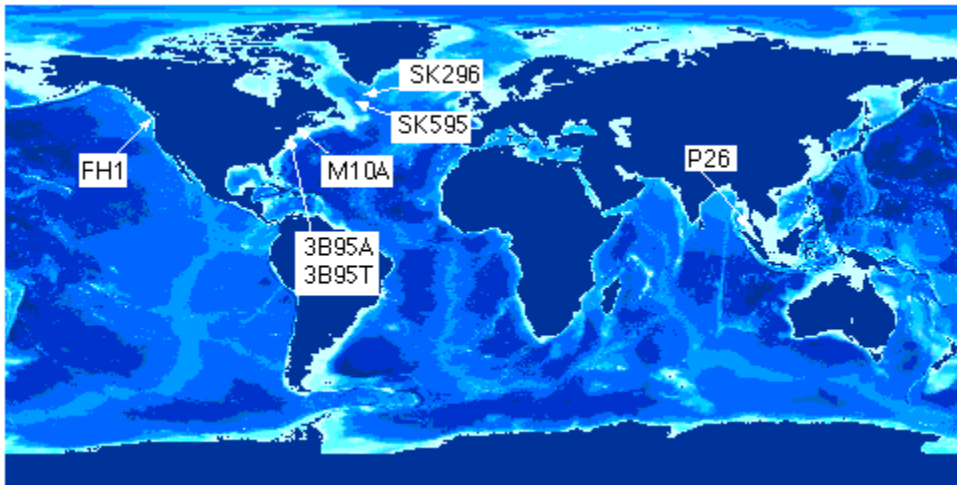


Fig. 1. Isolates of *C. contortus* used in the present study and their source locations.

In addition, different species and families of diatoms have been isolated to provide an out group comparison for the *C. contortus* isolates. A satisfactory technique for the extraction of genomic DNA has been developed and the DNA has been extracted from the isolates and stored at -70°C . Experimentation with a variety of restriction enzymes was done to find the enzymes that cut efficiently for this material. Techniques for Northern blotting of the DNA from agarose gels have been successfully implemented

and probing of the DNA on the blots has been started with probes from outside laboratories.

RESULTS

There is now sufficient diversity in the extracted DNA and enough material for many analyses of the type planned for this project as well as for future analyses beyond the scope of the present support. Thus far, several restriction enzymes have been found to cut the DNA from the isolates effectively. These include both 5 cutters (Eco RI and Hind III) and 4 cutters (Sma I and Dpn I). In each case, the restriction digest was best carried out under the manufacturers suggested overdigest conditions. These results are now being used in the preparation of the blots that will be probed to complete the RFLP analysis.

IMPACT

We now have some confidence that the existing diatom probes are a useful method for the analysis of *Chaetoceros* genetic patterns. When the complete suite of between- and among-species RFLP patterns are available, a method for quantitatively evaluating the dependence of open ocean blooms and their significant impact on the surface optical fields will be possible.

TRANSITIONS

There is no current effort to transfer these methods directly to Naval use. Depending on the outcome of the dispersion study, it may be possible to develop a reliable at sea method for determining the biological capacity of various water masses to form blooms.

RELATED PROJECTS

The probing of the DNA samples is being done in collaboration with of Dr. John Paul from the University of South Florida.

REFERENCES